WHO global action plan for laboratory containment of wild polioviruses

Second Edition

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Purpose

To provide a systematic global
plan of action to minimize the risk of reintroduction of wild polioviruses
from the laboratory into the community.

Executive Summary

The world will be declared polio-free when the World Health Organization (WHO) Global Commission for the Certification of the Eradication of Poliomyelitis (GCC) is satisfied that all Regions have documented the absence of wild poliovirus transmission for at least 3 consecutive years and that laboratories identified with wild poliovirus materials have implemented appropriate containment conditions.[1] The probability of wild poliovirus transmission from the laboratory to the community is small, but the consequences grow greater as polio-free countries increase in number and immunization decreases or stops. Safe handling and, ultimately, appropriate laboratory containment of wild poliovirus infectious and potential infectious materials is crucial.

The first edition of the *Global Action Plan for Laboratory Containment of Wild Polioviruses* was published by WHO in December 1999. The Plan was based on broad input from biosafety experts, epidemiologists, laboratory scientists, ministries of health of all nations, and vaccine manufacturers.

This second edition of the *Global Action Plan* replaces the first. It incorporates lessons learned from biomedical laboratory surveys and inventories in more than a hundred nations in five of the six WHO Regions. It expands previous recommendations to include vaccine-derived polioviruses (VDPV). It defines biosafety requirements in terms of risks. It describes two phases of activities leading to containment: the Laboratory Survey and Inventory Phase and the Global Certification Phase. Finally, it examines the implications of future global immunization policy decisions on containment in the Post Global Certification Phase.

Laboratory Survey and Inventory

This phase covers the period when the numbers of polio-free countries and Regions are increasing, but wild polioviruses continue to circulate somewhere in the world. During this phase, nations:

1. Survey all biomedical laboratories to identify those with wild poliovirus infectious or potential infectious materials and encourage destruction of all unneeded materials.
2. Develop an inventory of laboratories that retain such materials and report to the Regional Certification Commission.
3. Instruct laboratories retaining wild poliovirus infectious or potential infectious materials to institute enhanced biosafety level-2 (BSL-2/polio) measures for safe handling (**Annex 3**).
4. Plan for Global Certification.

Global Certification

This phase begins when one year has elapsed without isolation of wild poliovirus anywhere in the world. During this phase nations:

1. Notify biomedical laboratories that poliovirus transmission has been interrupted.
2. Instruct laboratories on the National Inventory to elect one of the following three options:
    - Render materials non-infectious for poliovirus or destroy them under appropriate conditions (**Annex 2**).
    - Transfer wild poliovirus infectious and potential infectious materials to laboratories capable of meeting the required biosafety standards.

- Implement biosafety requirements appropriate for the laboratory procedures being carried out (BSL-2/polio or BSL-3/polio).

3. Document completion of all containment requirements for global certification.

Post Global Certification

The global certification containment requirements are anticipated to remain in force with concurrent immunization policies. At sometime in the future, international advisory bodies are expected to re-examine post certification immunization policies in light of current research outcomes, national experiences, and assurances that surveillance, vaccine stockpiles, and emergency response plans are adequate should polio re-emerge. If universal polio immunization is discontinued, containment requirements for wild as well as OPV viruses are likely to become more stringent than outlined in this document, consistent with the consequences of inadvertent transmission of poliovirus from the laboratory to an increasingly non-immune community.

Publication of the plan

This document provides the background, rationale, and strategy to ensure that laboratory facilities and biosafety practices are consistent with the risk of inadvertent transmission of poliovirus to the community. Full cooperation and commitment at all levels are essential to ensure that polio will not be a threat to future generations.

Poliomyelitis

Description

Polio, or poliomyeliti*s*, is an infectious disease caused by poliovirus, a member of the genus *Enterovirus*. There are three serotypes of poliovirus: 1, 2, and 3. Susceptible human cells have a specific protein receptor to which poliovirus may attach and thereby enter. The virus infects cells of the oropharynx, the tonsils, the lymph nodes of the neck, and the small intestines. Infection progresses through cycles of virus replication. Once infection is established in the gastrointestinal tract, poliovirus can invade the central nervous system (CNS) by penetrating the blood/brain barrier or by spreading along nerve fibres.

When non-immune persons are infected with poliovirus, the outcome may vary from unapparent infection without symptoms to mild illness, aseptic meningitis, or paralytic poliomyelitis.[2] About 1% of infections result in recognized neurological illness. The incubation period is 4-35 days, typically 7-14 days. Initial clinical symptoms may include fever, fatigue, headache, vomiting, constipation, stiffness in the neck, and pain in the limbs. The virus multiplies and destroys motor neurons, which may result in a permanent paralysis of muscles activated by the affected nerves.

Mode of transmission

Poliovirus is transmitted from person to person either through droplets from the upper respiratory tract during the early days of infection or, more commonly, through ingestion of infectious faecal material in low hygiene situations.[3]

Poliovirus in nature

Following infection, poliovirus can be found in the oropharynx for one to two weeks, in the blood for about one week, and in faeces for one to two months, even in individuals without symptoms of disease. On autopsy of persons who have died of the disease, poliovirus has been recovered from faeces, intestinal contents, lymph nodes, brain tissue, and spinal cord tissue.

Less than 1% of infections result in poliomyelitis. Many “healthy” children shed virus during periods of high prevalence. Poliovirus in the environment is the direct result of recent poliovirus infections in the human community. Contamination of soil occurs through human defecation near dwellings, crop fertilization with untreated or inadequately treated night soil or sewage, and recycled wastewater for irrigation. Presence of poliovirus in sewage reflects the prevalence of infection in the community. Contamination of surface waters may occur through discharge of untreated or inadequately treated sewage or run off from contaminated soil.

Humans are the only natural reservoirs of poliovirus. Higher non-human primates (chimpanzees and gorillas) are susceptible to infection and disease, but these populations are not sufficiently large to sustain poliovirus transmission in the absence of human infections.[4]

Poliovirus survival

Poliovirus is resistant to inactivation by common laboratory disinfectants such as alcohol and cresols. The virus is rapidly destroyed by exposure to temperatures of 50^{o}C or greater, autoclaving, or incineration.[2] It is readily inactivated by dilute solutions of formaldehyde or

free residual chlorine, ultraviolet light, heat and drying. Inactivation is slowed by the presence of extraneous organic matter.

Under stable laboratory conditions poliovirus in clinical or environmental specimens may survive at freezing temperatures for many years, under refrigeration for many months, and at room temperatures for days to weeks.[2]

Rates of poliovirus inactivation in nature are greatly influenced by the immediate environment. Poliovirus infectivity has been estimated to decrease by 90% in soil every 20 days in winter and every 1.5 days in summer. A similar estimated 90% decrease at ambient temperatures occurs in sewage every 26 days, in freshwater every 5.5 days, and in seawater every 2.5 days.[4]

Polio vaccines

Protective immunity against poliomyelitis is conferred through immunization or natural poliovirus infection. Immunity is poliovirus serotype-specific. Protection against disease is associated with antibodies that circulate in the blood stream and prevent spread of the virus to the central nervous system. Protection against infection is associated with both circulating antibodies in the blood and secretory antibodies in the gut and upper respiratory tract.[5]

Both live attenuated oral polio vaccine (OPV) and the injectable inactivated polio vaccine (IPV) protect against paralytic poliomyelitis.[6, 7] However, neither vaccine provides absolute protection against infection or reinfection with the virus itself. IPV stimulates protective antibodies in the blood (i.e. circulatory immunity) that stops poliovirus in the gut from entering and replicating in CNS. IPV effectively prevents virus spread and controls polio in countries with good hygiene.[7] OPV, a live virus that replicates in the gut, additionally induces mucosal immunity that inhibits intestinal virus replication. The resulting reduction in faecal shedding is associated with decreased transmission to other persons, making the choice of OPV critical for the global polio eradication initiative.

However, the live OPV has been associated with vaccine associated paralytic poliomyelitis (VAPP) on the order of one in every 2.5 million doses administered.[7] In infrequent cases, with about a dozen identified worldwide, immunocompromised patients with B-cell deficiencies may continue to shed vaccine virus for extended periods, leading over time to an accumulation of genetic changes in the excreted virus.[8] Continuous person-to-person circulation of vaccine polioviruses over extended periods of time in poorly immunized populations may result in genetic changes where neurovirulence and transmissibility profiles are characteristic of wild poliovirus.[9] Such viruses pose risks similar to naturally occurring polioviruses.

Interruption of wild poliovirus transmission

Polio occurred worldwide prior to the advent of immunization in the mid-1950s. Immunization has been highly effective in reducing the number of cases worldwide.[10] Further reduction in highly endemic areas has been achieved through improved routine childhood immunization and the strategic use of OPV in the polio eradication initiative.[11] The interruption of wild poliovirus transmission is based on the assumption that wild poliovirus circulation will cease when it is deprived of its susceptible human host through immunization.[12] The continued decrease in the incidence of polio in many countries and the progressive disappearance of poliovirus genetic lineages suggest that the interruption of human-to-human transmission is achievable.

The Rationale for Containment

Less than one year after smallpox was eradicated in 1977, two cases occurred in the United Kingdom, both linked to a smallpox laboratory. The index case worked in a room located directly above the laboratory. Two persons died; the index patient as a result of infection, and the director of the laboratory, who took his own life because of the accident.[13] When polio is eradicated every effort must be made to ensure that wild poliovirus is not similarly transmitted from the laboratory to an increasingly non-immune community.

In theory, polioviruses may be transmitted to persons outside the laboratory through contaminated laboratory effluents released into sewage, solid wastes transported to landfills, spent air exhausted to surroundings, or through contaminated workers' skin or clothing. However, transmission through such routes is extremely difficult to document against a background of high levels of immunity acquired through natural infection or immunization.

More readily documented are poliovirus infections of laboratory workers with potential for transmission to the community. From 1941 to 1976 a total of 12 laboratory-associated poliomyelitis cases, including two deaths, were recorded.[14-17] Accounts of 7 of the 12 were unpublished. Most cases occurred in the pre-vaccine era and before the advent of cell culture.

The first report of a laboratory-associated infection, published in 1941, described a case of poliomyelitis most likely acquired through washing and grinding infected tissues in preparation for inoculation into monkeys.[18] Two years later, two laboratory workers were infected with the prototype Lansing (Armstrong) strain while attempting to infect mice.[19] Two additional reported cases of poliomyelitis in laboratory workers were fatal: one in the United States[20] and the second in South Africa.[21]

The paucity of reports of laboratory-associated poliomyelitis since vaccines were introduced testifies to the effectiveness of vaccines and vastly improved laboratory facilities, technologies, and procedures.[22, 23] Nevertheless, recent evidence indicates that the potential remains for transmission of poliovirus from the laboratory to the community. In 1992, a wild-type 1 strain used for IPV production was documented as being transmitted from a worker in a vaccine production facility to his young son.[24] In another incident, a child was reported infected with a prototype strain of type 3 commonly used in laboratories for research and IPV vaccine production. The source of this infection was not determined.

IPV is highly effective in preventing disease, but its use cannot be assumed to prevent silent infection among laboratory workers. OPV provides a more effective barrier but silent infections may still occur. The incidence of poliovirus infections without clinical symptoms among laboratory workers is unknown.

In the absence of fully effective vaccines, appropriate biosafety measures are crucial to prevent poliovirus infection of laboratory workers and subsequent transmission. Absolute containment cannot be assured. Questions of intentional or unintentional non-compliance will always remain. But effective containment, that is, reducing the risk of inadvertent reintroduction of wild poliovirus into the community, is a realistic goal.[25]

Definitions

Polioviruses (Box 1)

Polioviruses are defined by standard neutralization tests with specific antisera. The three poliovirus serotypes form a unique genetic group of human enteroviruses that initiate infection by binding to a specific cellular receptor (PVR:CD155). Other enteroviruses may occasionally be associated with cases of acute flaccid paralysis, but they are not polioviruses and they do not bind to PVR.

Wild polioviruses have the capacity to circulate indefinitely within susceptible human populations. Molecular studies have shown that the capsid sequence lineages of wild polioviruses are maintained along chains of transmission, while the noncapsid and noncoding sequences may be exchanged by recombination with other enteroviruses during circulation. Thus, the identification of sequences outside of the capsid region as "poliovirus" may be arbitrary.

OPV strains have been demonstrated by clinical studies to be significantly less neurovirulent than wild poliovirus and thus pose greatly reduced risks when administered to vaccinees. Candidate attenuated vaccine strains that have not undergone extensive field testing and have not been approved by national regulatory authorities for use in oral polio vaccines are regarded as wild polioviruses for purposes of containment.

Genetic mutations occur in all circulating polioviruses. Mutations in the VP1 region provide the basis for differentiating wild poliovirus isolates into genotypes and lineages. Mutations further characterize isolates of OPV origin. A difference in the range of 0-1% from the parent OPV strain by sequence homology of the full VP1 region is consistent with normal virus shedding or limited person-to-person spread. A difference in the range of 1-15% is characteristic of isolates from OPV-derived polio outbreaks, consistent with extensive transmission and the capacity to cause paralytic disease.[9]

Box 1: Definitions of polioviruses

Polioviruses: human enteroviruses that exist as three well-defined serotypes and infect cells via specific receptor PVR:CD155.

Oral poliovirus vaccine (OPV) strains: attenuated polioviruses approved for use in oral vaccines by national control authorities. Unapproved candidate strains are considered wild.

Wild polioviruses: field isolates known or believed to have circulated persistently in the community and reference strains derived from these isolates.

OPV-like polioviruses: field isolates consistent with a limited period of virus excretion or person-to-person transmission, usually demonstrating <1% difference from parent OPV strains by full VP1 sequence homology. Included are isolates that have not been sequenced but have been shown to be OPV-like by two WHO recommended methods of intratypic differentiation.

Vaccine-derived polioviruses (VDPV): field isolates consistent with an extensive period of virus excretion or transmission in the community, usually demonstrating 1-15% differences from parent OPV strains by full VP1 sequence homology. VDPVs are classified as wild for programmatic and containment purposes.

Materials are further categorized as wild poliovirus infectious or potential wild poliovirus infectious. Included in both of these categories are clinical and environmental materials and laboratory products of these materials. For purposes of containment, VDPV is considered wild.

Wild poliovirus infectious materials (Box 2)

Wild poliovirus (including VDPV) may be present in a variety of clinical materials, most commonly in faeces and throat specimens, less commonly in blood, and rarely in cerebrospinal fluids in non-paralytic and paralytic infections. In fatal infections, wild poliovirus may be present in faeces, intestinal contents, lymph nodes, brain tissue, and spinal cord tissue.[25] Poliovirus may be found in blood during the first week of infection, before neutralizing antibodies appear, but is rarely found in blood after onset of clinical signs of central nervous system involvement. All such clinical materials from persons with acute poliomyelitis are defined as infectious, even though the presence of virus may not have been confirmed.

Wild polioviruses present in environmental samples, such as sewage and water, reflect the presence of poliovirus in the community. The viral content of sewage may vary widely, depending on many environmental factors.

Infectious laboratory products include infected cell cultures, virus stocks, research materials where wild poliovirus has been used, and infected non-human primates and transgenic mice.[26]

Box 2: Wild poliovirus infectious materials are defined as:

Clinical materials from confirmed wild poliovirus and VDPV infections, environmental sewage or water samples in which such viruses are present, and replication products of such viruses, including

- Cell culture isolates, reference strains, seeds for inactivated vaccines
- Infected animals or samples from such animals, including PVR transgenic mice
- Derivatives produced in the laboratory that have capsid sequences from wild polioviruses
- Full length RNA or cDNA containing capsid sequences derived from wild poliovirus
- Cells persistently infected with poliovirus strains whose capsid sequences are derived from wild poliovirus

Potential wild poliovirus infectious materials (Box 3)

At least 99% of wild poliovirus infections cause no recognizable paralytic disease, but may result in significant numbers of wild polioviruses being shed in faeces and respiratory secretions. Wild poliovirus isolation rates of 8-19% have been reported from stools of healthy children during polio seasons in endemic areas.[27,28] Laboratories with stored collections of faecal, throat, or environmental samples should assess the likelihood of the presence of wild polioviruses in these materials, based on sample treatment and storage history, the country of origin, the year, and the time of the last indigenous wild poliovirus isolates in that country (see **Annex 1**). Uncharacterised enterovirus-like cell culture isolates or undifferentiated poliovirus isolates from such materials are included as potential wild poliovirus infectious materials until proven otherwise.[29] Frozen stool samples from young children during endemic periods are likely to have the highest levels of infectious wild polioviruses.

Box 3: Potential wild poliovirus infectious materials are defined as:

Faeces, respiratory secretions, and environmental sewage and water samples of unknown origin or collected for any purpose at a time and in a geographic area where wild polioviruses or VDPV were suspected to be present, as well as products of such materials in poliovirus permissive cells or animals, including:

- Harvests untested for polioviruses and enteroviruses
- Uncharacterised enterovirus-like cell culture isolates
- Undifferentiated poliovirus isolates

Serum samples and cerebrospinal fluids collected in polio endemic areas for other purposes are not considered as potential wild poliovirus infectious materials because of the low probability of infectious poliovirus being present.

Clinical or environmental materials stored without refrigeration for three months or more, refrigerated for one year or more, heat inactivated, treated with disinfectants known to inactivate polioviruses, or tested and found negative for the presence of enteroviruses are not considered infectious or potentially infectious for wild poliovirus.

Laboratory Survey and Inventory

This phase covers the period when the numbers of polio-free countries and regions are increasing but wild polioviruses continue to circulate somewhere in the world.
During this phase, nations:

1. *Survey all biomedical laboratories to identify those with wild poliovirus infectious or potential infectious materials and encourage destruction of all unneeded materials.*
2. *Develop an inventory of laboratories that retain such materials and report to the Regional Certification Commission.*
3. *Instruct laboratories retaining wild poliovirus infectious or potential infectious materials to institute enhanced biosafety level-2 (BSL-2/polio) measures for safe handling.*
4. *Plan for Global Certification.*

National, regional, and global inventories of all institutions/laboratories with stored stocks of wild poliovirus infectious or potential infectious materials provide the basis for achieving global laboratory containment when wild poliovirus transmission is interrupted. The four primary activities of this phase are described below.

1. Surveying laboratories

The purpose of the national survey is to identify all laboratories storing wild poliovirus infectious or potential infectious materials. A major function of the survey is to encourage destruction of those materials that are no longer needed. The national survey is hierarchical, beginning with notification to the national government by WHO and proceeding through Ministries of Health and other concerned Ministries to agencies and institutions, and to individual laboratories. Because many laboratories that might possess such materials are outside the health sector, completion of the national survey requires Ministries of Health to enlist the cooperation of other ministries, such as Education, Defence, and Environment (**Box 4**). Each country should designate a National Task Force/Coordinator for planning and implementing the multi-sectoral national survey and for verifying that all activities have been completed.

Many different types of laboratories may store wild poliovirus infectious or potential infectious materials. A variety of resources may be required to identify these laboratories, including national laboratory registries, accrediting bodies, professional organizations, and national and institutional biosafety networks.

Types of laboratories possibly storing wild poliovirus infectious materials are described below and summarized in **Box 4**.

> *Poliovirus/Enterovirus Laboratories:* Laboratories currently working with polioviruses, or those that have worked with polioviruses in the past, are likely sources of wild poliovirus materials. Such laboratories that conduct research or serve a diagnostic function are most likely found in universities or government health agencies.

General Virology Laboratories: Some virology laboratories, not necessarily identified as poliovirus laboratories, may work with wild polioviruses/enteroviruses or may have worked with such viruses in the past for diagnostic testing, research, or teaching exercises. Diagnostic and public health laboratories may have stored poliovirus isolates and clinical specimens from past investigations of endemic or imported cases of poliomyelitis. Some have multiple virus strains for test controls or reference purposes. Educational institutions may have wild polioviruses for teaching exercises. Virus research laboratories may have poliovirus stocks or infectious materials for studies on the biological, biochemical, or genetic properties of viruses. Such laboratories may be found in numerous organizations including public health institutions, national control agencies, clinical facilities, commercial services, research, and academic institutions.

Environmental Testing Laboratories: Some environmental laboratories may have wild poliovirus contaminated materials (sewage or water samples; see **Annex 1**) or wild poliovirus isolates as reference strains or controls.

Industry: Vaccine manufacturers have wild poliovirus strains for the production of IPV or often to test the quality of OPV. Such production laboratories are few in number and generally known to national regulatory authorities. Related companies, such as disinfectant manufacturers, may use wild poliovirus to measure the effectiveness of virucidal compounds or as reference standards.

Laboratories storing potential wild poliovirus infectious materials are more challenging to identify. These materials may include a variety of clinical or environmental samples collected for purposes unrelated to polio investigations. An example is a laboratory with faecal samples collected for diarrhoeal disease research during a time and in a geographical area of wild poliovirus endemicity.

All of the above-listed laboratories may have potential wild poliovirus infectious materials. Others include clinical bacteriology, parasitology, pathology, gastroenterology, and nutrition laboratories, which are likely to be located in hospitals (both private and government), academic institutions, and the private sector. Research laboratories studying enteric diseases, cholera, parasitic infections, or nutrition are of particular importance (**Box 4**).

Box 4 – Sectors, agencies/institutions, and laboratories that might possess wild poliovirus infectious or potential infectious materials

Types of sectors	**Types of agencies or institutions**	**Types of laboratories**
Health	Biological Standards/Control Agencies	Virology
Education	Biomedical Research Institutions	Bacteriology
Defense	Universities	Parasitology
Environment	Culture Collections	Gastroenterology
Agriculture	Environmental Agencies (water / sewage)	Pathology
Science and Technology	Hospitals / clinics	Molecular biology
Sectors unique to country structures	Military Agencies (health/research)	Nutrition
	Producers (biologic/vaccines/disinfectants)	Genetics
	Public Health Agencies	Environmental
	Agencies unique to country structures	Veterinary
		Medical

Each laboratory should conduct a thorough search for materials that meet the definition of wild poliovirus infectious or potential infectious materials. Laboratories should critically examine the need to retain any wild poliovirus materials and dispose of all such materials that serve no programmatic or research purpose. For most diagnostic tests, wild polioviruses may be replaced with OPV strains, inactivated antigens, or non-polio enteroviruses (**Annex 2**). If wild poliovirus materials are required, only viruses readily identifiable by molecular methods should be used. Laboratories retaining wild poliovirus infectious or potential infectious materials should be listed on the National Inventory and operate under Biosafety Level-2/polio (BSL-2/polio) conditions as described below.

2. Developing National Inventories

The purpose of the National Inventory is to document the location of the laboratory and types of wild poliovirus infectious or potential infectious materials being retained in the country, meet the country requirements for certification of Regions as polio-free, and maintain a current list of laboratories to be notified to initiate the appropriate containment procedures one year after detection of the last wild poliovirus.

The National Inventory is an active record maintained by the national government and updated regularly to prepare for the post global eradication phase. The National Inventory and supporting documents are prepared and presented to the National Certification Committee for review, endorsement, and submission to the Regional Certification Commission as a component of National Documentation for Certification of Polio Eradication.

National Inventories of laboratories with wild poliovirus infectious or potential infectious materials are compiled into Regional Inventories maintained by the WHO Regional Offices. Inventories from all six Regions constitute the Global Inventory maintained by WHO, Geneva, Switzerland.

3. Implementing Biosafety Level 2/polio

Laboratories listed on National Inventories as retaining wild poliovirus infectious or potential infectious materials should operate under Biosafety Level 2/polio (BSL-2/polio) conditions. The purpose of the BSL-2/polio requirement is to reduce the risk of reintroducing wild polioviruses from the laboratory into the community at a time when poliovirus circulation is decreasing or no longer occurring in many areas of the world. The designation BSL-2/polio refers to standard BSL-2 conditions in addition to specific requirements for wild polioviruses.

BSL-2 is defined as good microbiological practices in an appropriately equipped basic microbiology laboratory. Specific requirements are described in the 2002 WHO *Laboratory Biosafety Manual (3rd edition)*.[30] In brief, BSL-2 includes safe laboratory practices, appropriate disinfection, sterilization, and waste disposal procedures, and the availability and use of equipment designed to reduce or eliminate hazards. The basic microbiology laboratory consists of a facility with an autoclave on site and a certified class I or II biological safety cabinet or equivalent containment device for all manipulations with open infectious materials. A mechanical room ventilation system with inward directional airflow is desirable.

BSL-2/polio includes the following precautions specific for laboratories with wild poliovirus materials:

> *Operational practices:* Access to laboratories is restricted. All persons entering the laboratory, including support staff (cleaners, maintenance, etc.), are immunized with IPV or OPV, depending on national policy. Accurate records on wild poliovirus stocks are maintained. All manipulations with open wild poliovirus infectious or potential infectious

materials are performed using a class II biological safety cabinet or other primary containment device.

Storage: Wild poliovirus materials are stored in secure areas with limited access. Freezers are locked with limited access to the key mechanism. A detailed current inventory of all freezer contents and documentation of all withdrawals and additions is maintained. Stored wild poliovirus materials are clearly marked as such. Ideally, freezers in which such materials are stored are located within the BSL-2/polio laboratory or an equivalent facility.

Transfer of materials: Great care must be taken to avoid spills and breakages when transferring wild poliovirus infectious and potential infectious materials from the freezer. All are transferred in leak-proof, unbreakable secondary containers that can be disinfected if spillage should occur. Laboratories have standard operating procedures (SOPs) specifically for the safe transfer of materials to and from freezers. SOPs contain clear descriptions of procedures for response to all spillages, breakages and accidents that may occur when transferring materials.[31]

BSL-2/polio requirements are summarized in **Box 5** and described in detail in **Annex 3**.

Box 5: Summary of Biosafety Level 2/polio requirements

In addition to BSL-2 requirements, the BSL-2/polio facility incorporates the following standards:

Operational procedures

- Access to the laboratory is restricted.
- All persons entering the laboratory are fully immunized against polio.
- All manipulations with open wild poliovirus infectious or potential infectious materials are performed using a certified class II biological safety cabinet or other primary containment device

Storage

- Wild poliovirus infectious and potential infectious materials are stored in secure areas with limited access.
- Freezers and refrigerators are locked with limited access to the key mechanism and clearly marked as containing wild poliovirus materials
- Freezer inventories are current and complete, including nature of material, volume or amount, location in freezer
- Documentation is current on all materials, including geographical source and date of collection

Transfer of materials

- All materials are transferred to and from the freezer in leak-proof, unbreakable secondary containers
- Standard operating procedures (SOP) are established and regular training provided on responses to all spills, breakage of virus-containing vessels, and accidents where virus may have been released.

4. Preparing for Global Certification

Countries should establish channels for regular communications with laboratories on the National Inventory to periodically inform them of progress toward interruption of wild poliovirus transmission, the need to maintain updated inventories, and modifications in biosafety recommendations. The channels will be used later to notify laboratories of the effective date to implement appropriate biosafety measures. Countries electing to retain wild poliovirus infectious and potential infectious materials should begin now to ensure that the designated laboratories meet the appropriate biosafety requirements for facilities and staff training (**Annex 4**). Countries will have only one year after WHO notification to implement post global eradication requirements. Advanced preparations are advised.

Global Certification

This phase begins when one year has elapsed without isolation of wild poliovirus anywhere in the world. During this phase nations:

1. *Notify biomedical laboratories that poliovirus transmission has been interrupted.*
2. *Contact laboratories on the National Inventory and instruct them to elect one or more of the following three options:*
    - *Render materials non-infectious for poliovirus or destroy them under appropriate conditions.*
    - *Transfer wild poliovirus infectious and potential infectious materials to laboratories capable of meeting the required biosafety standards.*
    - *Implement biosafety requirements appropriate for the laboratory activities being performed (BSL-2/polio or BSL-3/polio).*
3. *Document completion of all containment requirements for global certification.*

The goal of this phase is to reduce the risk of wild poliovirus transmission from stored virus stocks and clinical materials at a time when universal immunization continues and wild polioviruses no longer circulate anywhere in the world.

1. Notifying laboratories when poliovirus transmission has stopped

WHO will notify all nations that wild poliovirus transmission has stopped when one year has passed with no evidence of wild poliovirus circulation anywhere in the world (Year 1). Nations will inform agencies/institutions and laboratories on the National Inventory that global certification biosafety requirements are to be implemented no later than one year from the date of the announcement (Year 2). During the third year nations will submit documentation to the Global Certification Commission that effective containment is in place (Year 3).

2. Implementing biosafety options

An underlying principle of poliovirus laboratory containment is that most laboratories do not have a need for long-term retention of wild poliovirus infectious and potential infectious materials. Destruction of such materials is strongly encouraged. Laboratories should critically evaluate the considerable personal and institutional responsibilities inherent in retaining a virus that is no longer being transmitted in nature.

Laboratories that do not implement the required containment conditions must render all wild poliovirus materials non-infectious, destroy by autoclaving or incineration (**Annex 2**), or transfer them to a laboratory that meets the appropriate containment level.

By definition, no clinical materials collected during the global certification phase are infectious for wild poliovirus, unless the virus re-emerges or VDPV is is detected. The threat of laboratory infection comes principally from stored materials collected before wild poliovirus transmission stopped . A small number of laboratories are expected to retain wild poliovirus materials for research purposes. Of particular importance is research relevant to defining the final strategy for stopping polio immunization.

Other research laboratories in larger institutions are anticipated to retain collections of potential wild poliovirus infectious materials valuable for studying other diseases. Laboratories retaining such materials should do an internal risk assessment (**Annex 1**) and implement biosafety measures appropriate for the laboratory activities being performed (**Box 6**). For containment purposes, samples are considered polio-free immediately after the date of the last documented

case for a given country. Experience has shown that widespread transmission ceases well before the last case is identified. The likelihood of collecting a virus positive specimen at random after that date is remote.

All wild poliovirus infectious materials (**Box 2**) must be handled under BSL-3/polio conditions. All activities with potential wild poliovirus infectious materials (**Box 3**) that involve inoculating poliovirus permissive cells or animals, that is, any biologic system in which polioviruses replicate, must also be performed under BSL-3/polio conditions.

All other activities involving potential wild poliovirus infectious materials may be handled safely within a certified class II biological safety cabinet inside a BSL-2/polio laboratory (**Box 5**). Potential wild poliovirus infectious materials may be centrifuged in the open laboratory if sealed heads or centrifuge safety cups are used and these are only opened within a biological safety cabinet.

Box 6: Global Certification Biosafety Requirements for Wild Poliovirus

Category of Material	Laboratory Activity	Biosafety Level
Wild poliovirus infectious	All activities, including storage	BSL-3/polio
Potential wild poliovirus infectious	Activities involving poliovirus permissive cells or animals	BSL-3/polio
	Other open activities involving stored clinical and environmental materials	BSL-2/polio, in class II biosafety cabinet

Implementing Biosafety Level 3/Polio: BSL-3 (the high containment laboratory) includes all BSL-2 requirements with additional emphasis on protecting personnel in adjacent areas, the community, and the environment. Specific requirements are defined for personal protective clothing, laboratory design, use of laboratory equipment, and medical surveillance of laboratory staff. The laboratory should be separated from high traffic areas of the building with access restricted to authorized personnel. Biosafety provisions must be made for air, water, and materials entering and leaving the laboratory, as described in the 2002 WHO *Laboratory Biosafety Manual (3rd edition)*.[30] The BSL-3/polio laboratory (**Box 7**) incorporates all BSL-3 practices, but specifies additional requirements for storage and transfer of wild poliovirus infectious materials (**Box 8**).

Box 7: Major features of biosafety levels 2/polio (Annex 3) and 3/polio (Annex 4)		
	BSL-2/polio	BSL-3/polio
Good microbiological technique	√	√
Personnel		
- Immunized	√	√
- Medical assessment		√
- Protective laboratory clothing	√	√
Facility		
- Separation of laboratory		√
- Restricted access	√	√
- Surfaces impervious to liquids	√	√
- Sealed for decontamination		√
- Inward directional airflow		√
- HEPA exhaust filters		√
- Biological safety cabinet I or II	√	√
- Autoclave on site	√	
- Autoclave in room		√
Wild Poliovirus Storage (see Box 8)	√	√
Listed on National Inventory	√	√

Implementing storage requirements: Poliovirus infectious materials stored under secure conditions pose no inherent risk of transmission. The risk emerges when these materials are removed from storage. Infectious materials are kept in locked freezers within the BSL-3/polio facility with restricted access. Potential wild poliovirus infectious materials are clearly marked as such, stored in locked freezers with restricted access, inventoried and documented. Ideally these freezers are located within laboratories having a BSL-2/polio facility.

As described in BSL-2/polio, a leak-proof, unbreakable secondary container should be used to avoid spills and breakages when transferring materials from freezers to safety cabinets. This practice is particularly important when potential wild poliovirus infectious materials are located in freezers outside the laboratory. Written laboratory procedures provide clear instructions for responding to spillages, breakages and accidents that may occur when transferring materials. Storage requirements are listed in **Box 8**.

Box 8: Global certification storage requirements for wild poliovirus infectious and potential infectious materials		
	Wild poliovirus infectious materials	**Potential wild poliovirus infectious materials**
Storage location	Secure area, ideally inside BSL-3/polio laboratory	Secure area inside facility
Documentation	Current documentation maintained on all materials including: • Geographical source and date of collection/isolation • Nature of collection source • Cell passage history • Genomic sequence of isolate • Complete composition, history, and properties of virus if a research product	Full and updated documentation maintained on all materials including: • Geographical source and date of collection/isolation • Nature of collection source
Security	Locked freezers with limited access to key mechanism	
Materials	Stored in leak proof, screw cap containers bearing a unique identifier number and name of responsible person	
Freezer Inventory	Full and updated inventory maintained including: • Nature of material • Volume or amount • Position in freezer	
Transfer of materials	• Leak-proof, unbreakable secondary containers • Specified procedures for response to spillage	

3. Documenting containment for Global Certification

Each Regional Certification Commission must submit satisfactory documentation to the Global Certification Commission that all laboratories in the Region with wild poliovirus infectious or potential infectious materials have either:

- Implemented appropriate biosafety conditions (BSL-2/polio or BSL-3/polio); or
- Transferred materials to WHO-designated repositories; or
- Rendered such materials non-infectious or destroyed them under appropriate conditions.[32]

Documentation should include:

- A current National Inventory of all laboratories retaining wild poliovirus infectious or potential infectious materials
- A quality assessment of the laboratory survey and inventory process.
- Evidence that laboratories retaining wild poliovirus infectious or potential infectious materials meet the required biosafety conditions

Detailed guidelines for assessing and documenting containment are scheduled for publication by WHO in early 2003.

Post Global Certification

This phase begins after the Global Commission has certified the world as polio free and international bodies have agreed on post certification immunization policies.[33,34] The four possible immunization scenarios include:

1) Continue OPV/IPV immunization either separately or in combination
2) Replace OPV immunization with IPV in all countries
3) Stop OPV immunization – countries may or may not elect to immunize with IPV
4) Stop all polio immunization

Global decisions on immunization policies will be based on outcomes of current research, post-eradication experiences, and assurances that surveillance, vaccine stockpiles, and emergency response plans are adequate if polio should re-emerge. If OPV immunization is stopped, laboratory containment requirements for wild as well as OPV viruses are anticipated to become more stringent than outlined in this document, consistent with the consequences of inadvertent transmission of poliovirus from the laboratory to an increasingly non-immune global community.

Sources

1 Department of Vaccines and Biologicals. Report of the third meeting of the Global Commission for the Certification of Eradication of Polio, July 9, 1998. Geneva, Switzerland: World Health Organization, 1999 (Reference WHO/EPI/GEN/981.17).

2 Melnick J. Enteroviruses: polioviruses, coxsackieviruses, echoviruses, and newer enteroviruses. Fields BN, Knipe DM, et al. Virology, 3rd Ed, Philadelphia: Lippincott-Rosen Publishers, 1996: 655-712.

3 Benenson A S., ed. Control of communicable diseases manual, 16th Ed. Washington, D.C.: American Public Health Association, 1995, 370.

4 Dowdle WR, Birmingham ME. The biologic principles of poliovirus eradication. The Journal of Infectious Diseases 1997, 175 (suppl 1): S286-92.

5 Ghendon Y, Robertson SE. Interrupting the transmission of wild polioviruses with vaccines: immunological considerations. Bulletin of the World Health Organization 1994, 72: 973-83.

6 Plotkin SA, Murdin A, Vidor E. Inactivated polio vaccine. Vaccines, 1999, 15: 345-63.

7 Sutter RW, Cochi SL, Melnick JL. In: Plotkin SA, Orenstein WA, editors. Vaccines. Philidelphia: W.B. Saunders; 1999. p. 365 – 408.

8 Kew O, Sutter R, Nottay B, et al. Prolonged replication of a type 1 vaccine-derived poliovirus in an immunodeficient patient. Journal of Clinical Microbiology. 1998, 36: 2893-2899.

9 Kew, O. M., V. Morris-Glasgow, M. Landaverde, C. Burns, J. Shaw, Z. Garib, J. André, E. Blackman, C. J. Freeman, J. Jorba, R. Sutter, G. Tambini, L. Venczel, C. Pedreira, F. Laender, H. Shimizu, T. Yoneyama, T. Miyamura, H. van der Avoort, M. S. Oberste, D. Kilpatrick, S. Cochi, M. Pallansch, and C. de Quadros. Outbreak of poliomyelitis in Hispaniola associated with circulating type 1 vaccine-derived poliovirus. Science, 2002, 296, 5566: 356-359.

10 World Health Organization. Progress towards the global eradication of poliomyelitis, 2001. Weekly Epidemiological Record, 2002, 13: 98-107.

11 World Health Organization. Expanded Program on Immunization – poliomyelitis eradication: the WHO Global Laboratory Network. Weekly Epidemiological Record, 1997, 245.

12 Eichner M, Dietz K. Eradication of poliomyelitis: when can one be sure that poliovirus transmission has been terminated? American Journal of Epidemiology. 1995, 143: 816-22.

13 Her Majesty's Stationery Office. Report on an investigation into the cause of the 1978 Birmingham smallpox occurrence. London: Her Majesty's Stationery Office, 1980.

14 Sulkin SE, Pike RM. Survey of laboratory-acquired infections. American Journal of Public Health and The Nation's Health, 1951, 41: 769-81.

15 Pike RM, Sulkin SE, Schulze ML. Continuing importance of laboratory-acquired infections. American Journal of Public Health, 1965, 55: 190-9.

16 Pike RM. Laboratory associated infections: summary and analysis of 3921 cases. Health Laboratory Science 1976; 13: 105-14.

17 Pike RM. Laboratory-associated infections: incidence, fatalities, causes and preventions. Annual Review of Microbiology, 1979, 33: 5.

18 Sabin AB, Ward RL. Poliomyelitis in a laboratory worker exposed to the virus. Science, 1941, 94: 113-4.

19 Beller K. Laboratoriumsinfektion mit dem Lansing-Virus. Zentralblatt fuer Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene Abt. 1 Orig., 1949, 153: 269-275.

20 Wenner HA, Paul JR. Fatal infection with poliomyelitis virus in a laboratory technician. American Journal of Medical Science, 1947, 213: 9-18.

21 Gear JHS, Rodger LM. Poliomyelitis in northern Rhodesia with special reference to an outbreak occurring on the Roan Antelope Copper Mine, Luanshya in 1946. South African Medical Journal, 1946, 20: 670-3.

22 Miller BM. (et al) Laboratory safety: principles and practices. Washington, D.C.: American Society for Microbiology, 1986, 322.

23 Sewell DL. Laboratory-associated infections and biosafety. Clinical Microbiology Review, 1995, 389-405.

24 Mulders MN, Reimerink JHJ, Koopmans MPG, van Loon AM, van der Avoort HGAM. Genetic analysis of wild type poliovirus importation into The Netherlands (1979-1995). Journal of Infectious Diseases, 1997, 176: 617-24.

25 Dowdle WR, Gary HE, Sanders R, van Loon AM. Can post-eradication laboratory containment of wild polioviruses be achieved? Bulletin of the World Health Organization, 2002, 80: 311-316.

26 World Health Organization. Maintenance and distribution of transgenic mice susceptible to human viruses: memorandum from a WHO meeting. Bulletin of the World Health Organization, 1993, 71: 497.

27 Tambini G, Andrus JK, Marques E, Boshell J, Pallansch M, de Quadros CA, Kew O. Direct detection of wild poliovirus circulation by stool surveys of health children and analysis of community wastewater. Journal of Infectious Diseases, 1997, 168: 1510-04.

28 Desphande JM, Kamat JR, Rao VK, Nadkarni SS, Kher AS, Salgaokar SD, Rodrigues JJ. Prevalence of antibodies to polioviruses and enteroviruses excreted by healthy children in Bombay. Indian Journal of Medical Research, 1995, 158: 707-12.

29 Pallansch M, Staples M. Wild poliovirus found in stored potential infectious materials. World Health Organization Polio Laboratory Network Quarterly Update, 2002, 8: 1-2.

30 World Health Organization. Laboratory biosafety manual, third edition. Geneva: World Health Organization. (www.who.int/emc-documents)

31 World Health Organization. Guidelines for the safe transport of infectious substances and diagnostic specimen. Geneva: World Health Organization,1997.

32 Global eradication of poliomyelitis: report of the third meeting of the Global Commission for the Certification of the Eradication of Polio. Geneva; World Health Organization: 1998 (unpublished document WHO/EPI/GEN/98.12; available from: URL:http//whqlibdoc.who.int/hq/1998/WHO_EPI-98.17.pdf).

33 Wood DJ, Sutter RW, Dowdle WR. Stopping poliovirus vaccination after eradication: issues and challenges. Bulletin of the World Health Organization, 2000, 78(3): 347-63

34 Technical Consultative Group on Global Eradication of Poliomyelitis. "Endgame" issues for the global polio eradication initiative. Clinical Infectious Diseases, 2002;34:72-77.

Annex 1: Year of Last Reported Indigenous Poliovirus Case by Country/Territory*

Country / Territory	Year of last polio	Country / Territory	Year of last polio	Country / Territory	Year of last polio	Country / Territory	Year of last polio
Afghanistan	Ongoing	Djibouti	1999[2]	Liberia	1999	Saint Helena	NR
Albania	1978	Dominica		Libyan Arab Jamahiriya	1991[1]	Saint Kitts & Nevis	1969[1]
Algeria	1996	Dominican Republic	1985[2]	Lithuania	1972[1]	Saint Lucia	1970[1]
American Samoa	1950s[1]	Ecuador	1990	Luxembourg	1963[1]	St. Vincent & Gren.	1977[1]
Andorra	1959[1]	Egypt	Ongoing	Macao, SAR	1975[1]	Samoa	1950s
Angola	Ongoing	El Salvador	1987[1]	Madagascar	1997	San Marino	1982
Anguilla		Equatorial Guinea	1992[1]	Malawi	1991[1]	Sao Tome & Principe	1983
Antigua & Barbuda	1965[1]	Eritrea		Malaysia	1985[2]	Saudi Arabia	1995
Argentina	1984[1]	Estonia	1961	Maldives	1980[1]	Senegal	1998
Armenia	1995	Ethiopia	Ongoing	Mali	1999	Seychelles	1980s[1]
Australia	1972[2]	Fed. S. of Micronesia	1970s[1]	Malta	1964[1]	Sierra Leone	1999
Austria	1980[1]	Fiji	1962[2]	Mariana Islands	1960s[1]	Singapore	1973
Azerbaijan	1995	Finland	1985	Marshall Islands	1976[1]	Slovakia	1960
Bahamas	1967[1]	TFYR Macedonia	1987[1]	Martinique		Slovenia	1978[1]
Bahrain	1993[1]	France	1989	Mauritania	1999	Solomon Islands	<1972
Bangladesh	2000	French Guiana	1983[1]	Mauritius	1980[1]	Somalia	Ongoing
Barbados	1967[1]	French Polynesia	1982[2]	Mexico	1990	South Africa	1989
Belarus	1964[1]	Gabon	1996[2]	Monaco	1964	Spain	1988
Belgium	1979[1]	Gambia	1997[2]	Mongolia	1993[2]	Sri Lanka	1993
Belize	1981[1]	Georgia	1991[1]	Montserrat		Sudan	Ongoing
Benin	2000	Germany	1990	Morocco	1989[1]	Suriname	1982[1]
Bermuda		Ghana	2000	Mozambique	1993[1]	Swaziland	1989[1]
Bhutan	1986[2]	Greece	1982	Myanmar	2000	Sweden	1977
Bolivia	1989	Grenada	1970[1]	Namibia	1995	Switzerland	1982
Bosnia & Herzogovinia	1961[1]	Guadeloupe		Nauru	1910[1]	Syrian Arab Republic	1998
Botswana	1989[1]	Guam	1964[1]	Nepal	2000	Tajikistan	1997[2]
Brazil	1989	Guatemala	1990[1]	Netherlands	1993	Thailand	1997
British Virgin Islands		Guinea	1999	Netherlands Antilles		Togo	1999
Brunei Darussalam	1978[2]	Guinea-Bissau	1999	New Zealand	1962[1]	Tokelau	1950s[1]
Bulgaria	1982	Guyana	1962	New Caledonia	1982	Tonga	1982[1]
Burkina Faso	2000	Haiti	1989[1]	Nicaragua	1981[1]	Trinidad & Tobago	1972
Burundi	1999[2]	Honduras	1989[1]	Niger	Ongoing	Tunisia	1994
Cambodia	1997	Hong Kong, SAR	1983	Nigeria	Ongoing	Turkey	1998
Cameroon	1999	Hungary	1969	Niue	1950s[1]	Turkmenistan	1996
Canada	1979	Iceland	1960[1]	Norway	1969	Turks & Caicos Islands	1977
Cape Verde	1988[1]	India	Ongoing	Oman	1993[1]	Tuvalu	1936[1]
Cayman Islands	1958[1]	Indonesia	1995	Pakistan	Ongoing	Uganda	1996
Central African Republic	2000	Iran (Islamic Rep.)	1997	Palau	1940s[1]	United Kingdom	1982
Chad	2000	Iraq	2000	Palestine N.A.	1988	Ukraine	1996[2]
Chile	1975	Ireland	1965[1]	Panama	1972[1]	United Arab Emirates	1992[1]
China	1994	Israel	1988	Papua New Guinea	1996[1]	United Rep. Tanzania	1996
Colombia	1991	Italy	1982	Paraguay	1985[1]	USA	1979
Comoros	1983[1]	Jamaica	1982	Peru	1991	Uruguay	1978[1]
Congo	2000	Japan	1980	Philippines	1993	US Virgin Islands	
Cook Islands	1959	Jordan	1988[1]	Poland	1984	Uzbekistan	1995
Costa Rica	1972	Kazakhstan	1995[1]	Portugal	1986	Vanuatu	1989[2]
Cote d'Ivoire	2000	Kenya	1988[1]	Puerto Rico		Venezuela	1989
Croatia	1990	Kiribati		Qatar	1990[1]	Viet Nam	1997
Cuba	1962[1]	Kuwait	1985[1]	Republic of Korea	1983[2]	Wallis and Futuna	1972[2]
Cyprus	1995	Kyrgyzstan	1993	Republic of Moldova	1991[2]	Yemen	1999[2]
Czech Republic	1960	Latvia	1962[1]	Reunion	1979[1]	Yugoslavia	1996
DPR Korea	1996	Lao PDR	1996	Romania	1992	Zambia	1995
Denmark	1976	Lebanon	1994[1]	Russian Federation	1996[2]	Zimbabwe	1991[2]
Dem. Rep. of the Congo	2000	Lesotho	1987[1]	Rwanda	1999[2]		

*The year of the last indigenous virologically confirmed case is used - cases due to imported wild poliovirus or circulating vaccine derived poliovirus are not reflected on this table. "Ongoing" refers to countries still considered endemic for wild poliovirus in 2001 (shaded).

[1] Details about case are not known;

[2] Clinically confirmed case;

Annex 2. Methods for disposal of wild poliovirus infectious or potential infectious materials[30]

Sterilization (use of autoclaves)

Moist steam under pressure is the most effective method of sterilization of laboratory materials.

- All cultures and contaminated materials should normally be autoclaved in leak-proof containers, e.g. autoclavable, colour-coded plastic bags, before disposal.
- Packaging should allow for penetration of steam.
- After being autoclaved, the materials may be placed in transfer containers for transport to the point of disposal.
- The autoclave should be validated to ensure sterilizing conditions are being met under all loading patterns.

Incineration

Incineration is the method of choice for final disposal of contaminated waste, including carcasses of laboratory animals, preferably after autoclaving. Incineration of infectious materials is an alternative to autoclaving only if:

- The incinerator is under laboratory control;
- The incinerator is provided with an efficient means of temperature control and a secondary burning chamber.

Final disposal

The disposal of laboratory and medical waste is subject to various national regulations. In general, ash from incinerators may be treated in the same way as normal domestic waste and removed by local authorities. Autoclaved waste may be disposed of by off-site incineration or in licensed landfill sites.

Annex 3: BSL-2/polio biosafety requirements

The BSL-2/polio facility consists of the following:

Physical Facilities

- The international biohazard sign with emergency contact information is displayed on the doors of the rooms where poliovirus is handled
- The laboratory is designed to facilitate cleaning and disinfection. Interior surface coatings (i.e. floors, walls, ceilings) are impervious to liquids and chemicals.
- Hand wash basins, preferably "hands-free" are provided in each laboratory room, preferably near the exit door.
- Backflow prevention is provided on services entering the laboratory (e.g. water supply)
- A class II biological safety cabinet or equivalent primary containment device is in the laboratory
- An autoclave is available in the same building as the laboratory
- Facilities for storing outer garments and personal items are outside the working areas
- Facilities for eating and drinking and for rest are outside the working areas
- Safety systems cover fire, electrical emergencies, emergency shower, and eyewash facilities
- First aid areas or rooms are suitably equipped and readily accessible

Operational Practices

- Access to the laboratory is restricted to authorized personnel
- A documented procedural manual for the BSL-2/polio laboratory outlining the safety practices and emergency procedures is written and followed.
- All personnel are trained on the operational procedures, the physical design features of the facility, the practices to prevent the release of infectious agents from the facility and emergency practices.

- All staff members working in the laboratory are immunized with IPV or OPV, depending on national policy.
- Staff members entering the laboratory wear protective clothing (gowns, coveralls, gloves) dedicated to the area.
- Hands are washed after handling infectious materials, after removing gloves, and when leaving the laboratory.
- Eating, drinking, smoking, applying cosmetics, and handling contact lenses is prohibited in the laboratory work areas
- Work surfaces are decontaminated with a disinfectant effective against poliovirus each day and after any spill of viable material.
- Good microbiological practices designed to minimize contact with infectious agents are followed.
- Pipetting by mouth is forbidden
- All laboratory technical procedures are performed in a way that minimizes formation of aerosols and droplets
- All manipulations with open infectious materials are conducted in a certified class II biological safety cabinet or other primary containment device
- All accidents, overt or potential exposures to infectious materials, breaches of containment and other hazardous occurrences are reported immediately to the laboratory supervisor.
- All laboratory waste is decontaminated by autoclaving or incineration
- Wild poliovirus infectious and potential infectious materials are stored in secure areas with limited access

- Freezers and refrigerators are clearly marked as containing wild poliovirus materials
- Freezer inventories are current and complete, including nature of material, volume or amount, and location in freezer
- Documentation is current on all materials, including geographic source and date of collection
- All materials are transferred to and from the freezer in leak-proof unbreakable secondary containers
- Standard operating procedures (SOP) are established and regular training on responses to all spills, breakages of virus-containing vessels, and accidents where virus may have been released.

Annex 4. BSL-3/polio containment requirements.

In addition to BSL-2/polio the BSL-3/polio facility consists of the following:

Physical Facilities

- The laboratory is separated from areas that are open to unrestricted traffic flow within the building.
- Office areas are located outside of the laboratory.
- Doors to the laboratory are labelled as a BSL-3/polio facility, requiring polio immunization by all persons entering the laboratory.
- Passage through a double-door entry with an area designed to don protective clothing dedicated to the polio laboratory is required for entry into the laboratory from access corridors.
- Penetrations in the containment barrier are sealed to facilitate cleaning and decontamination of the area.
- Windows are resistant to breakage and sealed shut.
- A hand washing sink with hands-free capability is provided near the exit door.

- A controlled ventilation system is installed to maintain inward directional airflow. Visual monitoring devices and/or alarm systems confirming directional airflow are used. Supply and exhaust air systems are interlocked to prevent positive pressurization of the laboratory
- Exhaust air is HEPA filtered depending on location of discharge. The exhaust air is not recirculated to other areas unless it has passed through HEPA filtration.
- Lengths of contaminated ventilation ductwork are minimized and sealed.
- An autoclave or other appropriate means for decontamination of infected waste in the containment barrier is provided in the laboratory.
- The performance of containment components and equipment are verified prior to operation and re-verified annually.

Operational Practices

- A documented procedural manual for the BSL-3/polio laboratory outlining the safety and containment practices is written and followed. Emergency procedures (SOP) are also written.
- All personnel are trained on the operational procedures, the physical design features of the facility, the practices to prevent the release of infectious agents from the facility and emergency practices.
- Staff members working in the BSL-3/polio laboratory are enrolled in a health and medical surveillance programme and are immunized with OPV or IPV according to national policy.
- All protective clothing is removed before exiting the laboratory and is decontaminated before reuse or disposable clothing is used and discarded as contaminated laboratory waste.
- Equipment is decontaminated prior to removal from the laboratory.
- An insect and rodent control programme is in effect.
- Storage requirements for wild poliovirus infectious and potential infectious materials are followed (**Box 8**)